DOCKET NO.: SNL-0004 / SD-8433 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **West, *et al.***	Confirmation No.: **4414**
Serial No.: **10/701,097**	Group Art Unit: **1744**
Filing Date: **November 4, 2003**	Examiner: **Bowers, Nathan A.**

For: **MICROFLUIDIC INTEGRATED MICROARRAYS FOR BIOLOGICAL DETECTION**

DATE OF ELECTRONIC DEPOSIT: May 15, 2009

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Commissioner for Patents
P.O. Box 1450
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Sir:

APPELLANTS' BRIEF PURSUANT TO 37 C.F.R. § 41.37

This brief is being filed in support of Appellant's appeal from the final rejections of claims 1-114, dated June 20, 2008. A Notice of Appeal was filed on November 20, 2008, and a decision on a request for pre-appeal brief conference was mailed on April 15, 2009.

1. REAL PARTY IN INTEREST

The real party in interest is Sandia National Laboratories, a facility owned by the U.S. government and operated at least in part by contractors, the facility having locations in Albuquerque, NM, and Livermore, CA. The present application has been licensed by Arcxis Biotechnologies, Inc., a U.S. corporation having its principal office in Pleasanton, CA.

2. RELATED APPEALS AND INTERFERENCES

No related appeals or interferences are pending.

3. STATUS OF CLAIMS

Pending:	Claims 1-114
Rejected:	Claims 1-114
Objected to:	None
Allowed:	None
Withdrawn:	None
Appealed:	Claims 1-114
Appeal Withdrawn:	None

4. STATUS OF AMENDMENTS

No claim amendments were filed subsequent to the final rejection dated June 20, 2008.

5. SUMMARY OF CLAIMED SUBJECT MATTER

The following summary is for the purpose of complying with the provisions of 37 C.F.R. § 41.37(c)(1)(v). The entire disclosure should be reviewed to obtain a complete understanding of the claim language. Citations to the specification are by paragraph number, e.g., "[0001]" and citations to the figures are by figure number, *e.g.*, "[Fig. 1, reference numeral 100]."

Claim 1	
Claim Language	Citation to specification and drawings
A microfluidic chip, comprising:	Abstract, [0008], [0009], [0031], for example
a plurality of vias;	[0008], [0011], [Fig. 1, reference numerals 20, 30, 40, 50, 60, 70, 80, and 90], for example
a functionalized porous polymer monolith capable of being in fluid communication with at least one of said vias;	Abstract, [0008], [0009], [0049], [0063], for example
a microarray capable of being in fluid communication with said functionalized porous polymer monolith;	Abstract, [0008], [0009], [0049], [0063], for example

the microarray situated within at least one microchannel, the microchannel being characterized as having depth in the range of from about 1 micron to less than 10 microns; and	[0056], [0068], [0070], [0087], for example
an observation port through which at least one target disposed within said microarray is capable of being detected.	Abstract, [0008], [0009], [0049], [0064], for example

6. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

- Claims 1-11, 55-64, 66, 68-77, and 111-114 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies (US 2004/0209354) in view of McNeely (US 2004/0037739) and Quake (US 6,833,242)
- Claims 16-25, 31-44, and 51-54 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake further in view of Schembri (US 6875620)
- Claims 12-15, 19, 25-35, 43-46, and 48-54 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake and further in view of Yamamoto (US 2004/0038388)
- Claim 65 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake as applied to claim 64 and further in view of Klaerner (US 2002/0001845)
- Claim 67 is rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake as applied to claim 62 further in view of Zare (US 2003/0062310)
- Claims 78-90, 93, 94, 96, 97, and 106-110 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake as applied to claim 1 and further in view of Werner (US 2002/0168652)
- Claims 91 and 98-105 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely , Quake and Werner as applied to claims 87 and 89, and further in view of Christel (US 6,368,871)
- Claims 92 and 95 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely, Quake, and Werner as applied to claims 90 and 93, and further in view of Regnier (US 6,156,273)

7. ARGUMENT

At issue in this appeal is the rejection of Claims 1-11, 55-64, 66, 68-77, and 111-114 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies (US 2004/0209354) in view of McNeely (US 2004/0037739) and Quake (US 6,833,242). The other rejections in this matter will stand or fall based on resolution of this rejection.

A. Summary of Argument

The pending claims recite microfluidic chips having, *inter alia*, microarrays disposed within microchannels having depths of between about 1 to about 10 microns. These chips are useful in the analysis and identification of biological organisms, including organisms that may be used as bioweapons.

The pending claims stand finally rejected. But a review of the cited references and of the evidence in the record makes clear that (1) the rejections are in fact based upon references that teach away from combination with one another and from the claimed invention; and (2) the Examiner failed to consider Appellants' objective evidence of non-obviousness. The rejections should be reversed and all claims should be allowed.

First, the Examiner's proposed prior art combination is contrary to well-established patent law. After expressly conceding that combining the Mathies and McNeely references would not address every element of Appellants' claims, the Examiner sought to remedy the admitted shortcoming of the Mathies-McNeely combination by combining these references with the Quake reference. But because the Mathies-McNeely combination teaches away from both combination with Quake **and** away from the claimed invention, the Mathies-McNeely-Quake combination violates established obviousness law and cannot support a *prima facie* case of obviousness.

Further, even if the Examiner's reference combination were proper, Appellants have provided objective evidence that the performance of the claimed invention is superior to the prior art and to existing technologies by **two orders of magnitude**, and evidence of this degree is sufficient to overcome a *prima facie* case of obviousness. The Examiner, however, failed to fully consider this objective evidence, despite the fact that this evidence establishes the non-obviousness of the pending claims.

As explained in *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992) (citations omitted):

> [T]he examiner bears the initial burden, on review of the prior art or on any other ground, of presenting a *prima facie* case of unpatentability. If that burden is met, the burden of coming forward with evidence or argument shifts to the applicant.
>
> After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of argument.
>
> If examination at the initial stage does not produce a *prima facie* case of unpatentability, then without more the applicant is entitled to grant of the patent.

Thus, the Board must first determine whether the Examiner has established a *prima facie* case of unpatentability before turning to Appellants' position on appeal, for "without more[,] [Appellants] are entitled to grant of the patent." *Id.* Appellants submit that review of the Examiner's arguments set forth in the Office Action demonstrates that the Examiner failed to establish a *prima facie* case of unpatentability. Further, assuming *arguendo* that the Examiner did establish a *prima facie* case of unpatentability, a point Appellants do not concede, Appellants submit that the Board's review of the totality of the record and of the relative persuasiveness of the parties' arguments will nonetheless demonstrate the patentability of the pending claims.

B. Legal Standard

As set forth above, the Examiner bears the initial burden of presenting a *prima facie* case of unpatentability. *In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992). If that burden is met, the burden of coming forward with evidence or argument shifts to the applicant. *Id.* If the Examiner does not establish a *prima facie* case of unpatentability, then the applicant is entitled to grant of the patent. *Id.*

"[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1739-43 (2007) (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). There must also be a reasonable expectation of success, and the cited prior art must address every element of the claim at issue. The rationale to make the claimed

combination and the reasonable expectation of success must be found in the prior art and not rest on impermissible hindsight based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

Once the Examiner makes out a *prima facie* case of obviousness, the applicant may rebut that case with objective evidence of nonobviousness, such as unexpected results or superior performance over the prior art. *E.g.*, *Takeda Chem. Indus. v. Alphapharm Pty., Ltd.*, 492 F.3d 1350, 1362 (Fed. Cir. 2007). The Examiner must fully consider all such evidence that an applicant may present. *Stratoflex, Inc. v. Aeroquip, Inc.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983). The ultimate determination of patentability is based upon the record as a whole. *In re Oetiker*, 977 F.2d at 1445.

C. Separate Argument For Claim 1

1. The Examiner's Combination Of References Cannot Support A *Prima Facie* Case Of Obviousness Because The References Teach Away From Their Combination And From the Claimed Invention

Although the Mathies, McNeely, and Quake references expressly teach away from each other as well as away from the claimed invention, the Examiner nonetheless found that the pending claims were obvious in view of the combination of these three references (June 20, 2008 Office Action at 3). But because the Examiner's reference combination is contrary to established patent law, the rejection of claim 1 is improper and should be vacated.

It is a fundamental principle of patent law that an obviousness rejection may not be based on a combination of references that teach away from their combination. *E.g.*, *Ecolochem, Inc. v. Southern Cal. Edison Co.*, 227 F.3d 1361, 1373-75 (Fed. Cir. 2000) (reversing district court's finding of obviousness where cited references taught away from their combination); *In re Grasselli*, 713 F.2d 731, 743 (Fed. Cir. 1983) (references that taught away from their combination cannot be combined to support obviousness rejection). References that teach away from the claimed invention likewise cannot support an obviousness rejection. *See W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1550 (Fed. Cir.) (reversing district court's finding of obviousness where district court erred by "disregarding disclosures in the references that diverge from and **teach away from the invention at hand**") (emphasis added), *cert. denied*, 469 U.S. 851 (1984). Despite this clear guidance from the Federal Circuit, the Examiner nonetheless combined references that teach away from one another **and** from the claimed invention.

Appellants' claim 1 recites, *inter alia*, "[a] microarray situated within at least one microchannel, the microchannel being characterized as having depth in the range of from **about 1 micron to less than 10 microns**." The Examiner candidly acknowledged that "[t]he combination of Mathies and McNeely [ ] **fails to disclose Applicant's claimed invention because neither reference teaches the use of microchannels having depths in the range of 1 to 10 microns**" (June 20, 2008 Office Action at 3) (emphasis added). In an effort to address Appellants' claim limitation regarding microchannels of about 1 to about 10 microns in depth, the Examiner relied on Quake for that reference's mention of microchannels having a diameter of 2 to 5 microns (June 20, 2008 Office Action at 5, 21). But a careful review of the references reveals that the Examiner's reliance on Mathies, McNeely, and Quake is improper as a matter of Federal Circuit law.

More specifically, the McNeely reference teaches that microarray-containing channels be "**at least about 15 μm** [microns]" in depth (McNeely at [0084]) (emphasis added), and are even more preferably more than about 25 μm in depth. This teaching away is further underscored by the statement in McNeely that if the chamber goes below a certain depth, "the chamber may become too difficult to fill . . . reducing the volume by **reducing the chamber height causes problems if the height goes below about a certain height** [*i.e.*, about 15 microns]" (McNeely at [0084]) (emphasis added). By contrast, Quake recites channels that are **2-5 microns** in depth (Quake at col. 24, lines 49-49). Thus, Quake's channels of **2-5 microns** in depth are plainly contrary to McNeely's teaching that channels be **more than 15 microns** in depth, and the case law is clear that it is improper to combine references where the references teach away from their combination, *Ecolochem*, 227 F.3d at 1373-75, which is precisely the situation the Examiner created in this case. Accordingly, because the references teach away from their combination, the McNeely-Quake-Mathies combination is improper as a matter of Federal Circuit law and cannot support the proposed obviousness rejection. *Id.*; *In re Grasselli*, 713 F.2d at 743.

Further, in addition to teaching away from combination with the other references, McNeely reference's requirement that channels be **more than 15 microns in depth** also teaches away from Appellants' claim 1 recitation that the channel depth be in the range of from **about 1 micron to less than 10 microns**. References that teach away from the claimed invention are also improper bases for an obviousness rejection. *See W.L. Gore*, 721 F.2d at 1550 (reversing district court's finding of obviousness where district court erred by "disregarding disclosures in the references that diverge from and **teach away from the**

invention at hand"). Accordingly, the Examiner's combination is also improper because it teaches away from the claimed invention. *Id.*

Ignoring the Federal Circuit's prohibition on combining references that teach away from their combination and also from the claimed invention, the Examiner attempted to sidestep binding precedent by stating that "McNeely is not relied upon for teachings regarding channel dimension, but is merely cited for motivation regarding the use of microarrays during detection" (June 20, 2008 Office Action at 21). The Examiner then went on to suggest – without citation to **any** record evidence – that "[i]t is well within the purview of one of ordinary skill in the art to weigh" the advantages the channels disclosed by Quake against the disadvantages of the channels described by McNeely (June 20, 2008 Office Action at 20). But this approach is contrary to Federal Circuit precedent.

An Examiner may not selectively rely on only those parts of a reference he believes are most helpful to his position without regard to the remainder of the reference. Instead, a prior art reference must be considered in its entirety, *i.e.*, as a whole, including portions that would lead away from the claimed invention. *See W.L. Gore*, 721 F.2d at 1550 (reversing district court's finding of obviousness where district court erred by "considering the references in **less than their entireties**"). Further, an Examiner's assessment of what is known in the art must be based on citation to record evidence, not on mere speculation. *See In re Zurko*, 258 F.3d 1379, 1385 (Fed. Cir. 2001) (assessment of what is known in the art must be based on citation to record evidence).

Here, the Examiner failed to confront the fact that McNeely teaches away from both the claimed invention and from combination with Quake, which violates the Federal Circuit's mandate that references must be considered in their entireties. *W.L. Gore*, 721 F.2d at 1550. Further, the Examiner provided no citation to the record to justify his speculation, and an Examiner's mere subjective belief – without **any** citation to concrete evidence in the record – is not a sufficient basis for an obviousness rejection. *See In re Zurko*, 258 F.3d at 1385 (assessment of what is known in the art must be based on citation to record evidence). Accordingly, the Examiner's justification for the Mathies-McNeely-Quake combination is based on no more than a hindsight rationale that selectively relies on only particular aspects of the cited references to arrive at the claimed invention, and does not explain why one of ordinary skill would ignore the references' teaching away from their combination.

In sum, the Examiner's analysis cannot be reconciled with the Federal Circuit's decisions in *Ecolochem* and *In re Grasselli*, both of which make clear that it is improper to

combine references where the references teach away from their combination. The Examiner's analysis also ignores the admonitions in *W.L. Gore* and *In re Zurko* that references must be considered in their entirety and that speculation regarding what is known in the art be must based on citation to record evidence. Accordingly, because the cited references teach away from combination with one another and from the claimed invention, the McNeely-Quake-Mathies combination is improper and can not support a *prima facie* case of obviousness. The obviousness rejections of the pending claims should be vacated and the claims should be passed to allowance.

2. Appellants Provided Sufficient Objective Evidence of Superior Results To Rebut A *Prima Facie* Case Of Obviousness

Even if the Examiner's proposed combination of references were proper and could support a *prima facie* case of obviousness, which Appellants do not concede, Appellants have in any event submitted sufficient evidence of secondary considerations to rebut that *prima facie* case of obviousness. *E.g.*, *Ashland Oil v. Delta Resins & Refractories*, 776 F.2d 281, 293 (Fed. Cir. 1985) ("[a]ll facts relevant to the issue of obviousness, both the facts established by the party asserting invalidity **and the facts established by the rebuttal evidence submitted by the patentee**, must be fully considered") (emphasis added).

More specifically, in the declaration submitted on March 25, 2008, Dr. Jason A.A. West, a co-inventor of the present invention, stated that the claimed invention "achieves results that are unexpectedly superior to those of alternative devices in the field," and Appellants supplied evidence – attached to that declaration – of the invention's unexpected superiority over existing devices and over the prior art. *E.g.*, *In re Geiger*, 815 F.2d 686, 690 (Fed. Cir. 1987) (evidence of superior performance sufficient to rebut *prima facie* case of obviousness); *In re Chupp*, 816 F.2d 643, 646-47 (Fed. Cir. 1987) (same).

A comparison between the claimed invention and the prior art was easily made and underscores the claimed invention's superior performance. The McNeely reference states that performing an analysis based on hybridization of probes to the microarrays disclosed in that reference includes, *inter alia*, an incubation period to allow the probes to hybridize to the array that is "typically performed **overnight**" (McNeely at [0153], emphasis added). By contrast, Exhibit B attached to Dr. West's March 25, 2008 declaration shows that the claimed invention achieves probe hybridization and detection in only about **5 minutes**. Conservatively assuming an 8-hour overnight incubation period, the claimed invention thus represents at least a **96-fold improvement** over the Examiner's cited prior art. Page 5 of

Exhibit C attached to Dr. West's declaration likewise establishes that devices according to the claimed invention are capable of performing probe hybridization and detection in 20-30 minutes, which is a fraction of the time required by the McNeely reference to accomplish the same operation. Given that the Federal Circuit has held that a **7-fold improvement** in performance over the prior art is sufficient to overcome a *prima facie* case of obviousness, Appellants' evidence establishes the claimed invention's nonobviousness. *Compare In re Wiechert*, 370 F.2d 927, 962 (C.C.P.A. 1967) (Rich, J.) (**7-fold** improvement of activity over the prior art sufficient to rebut *prima facie* case of obviousness); *In re Geiger*, 815 F.2d at 690; *Takeda Chem. Indus. v. Alphapharm Pty., Ltd.*, 492 F.3d 1350, 1355 (Fed. Cir. 2007) (superior performance relative to prior art rebutted *prima facie* case of obviousness).

The prior art and Appellants' evidence also establish that the claimed invention meets the art's long-felt need for rapid hybridization assays. Page 1 of Exhibit C to Dr. West's declaration states that "major drawbacks" to existing hybridization-based assays are the long sample processing time and the extended time requirement for probes to hybridize to target DNA on a slide surface. The McNeely reference cited by the Examiner likewise notes the "high level of interest" in developing rapid microarray-based assays (McNeely at [0005]). Thus, the objective evidence of record establishes that the claimed invention addresses this long-felt need in the art. *See Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

In the final rejection, the Examiner failed to provide any discussion of Dr. West's declaration, suggesting only that the declaration failed to provide a proper comparison between the invention and existing products. But this is not the proper standard. The law does not require that an applicant's evidence of secondary considerations make out a one-to-one comparison between the claimed invention and the prior art. Instead, an applicant may meet their burden by comparing the claimed invention with the closest subject matter that exists in the prior art. *In re Chapman*, 357 F.2d 418, 422 (C.C.P.A. 1966); *In re Geiger*, 815 F.2d at 689 (Newman, J., concurring); *see also* MPEP § 716.02(g). This is so because an applicant need not compare his or her invention with itself. *In re Chapman*, 357 F.2d at 422. Here, Appellants presented the Examiner with evidence that the claimed invention's performance was superior – by orders of magnitude – to that of the Examiner's own cited McNeely prior art, and the Examiner failed to provide any explanation for concluding that the claimed invention was obvious in view of this demonstrated superiority. The Examiner thus applied an incorrect legal standard and, moreover, failed to fully consider the import of this evidence. *See Stratoflex*, 713 F.2d at 1538 (reiterating that examiner must fully consider all

of an applicant's evidence of secondary considerations). Accordingly, Appellants' submissions clearly establish the secondary considerations of long-felt need in the art and the superiority of the claimed invention over existing devices and techniques, which objective evidence is sufficient to overcome a *prima facie* case of obviousness.

D. Separate Argument for Dependent Claims

For the purpose of this appeal, the patentability of the following claims will stand or fall together with claim 1:

1. Claims 16-25, 31-44, and 51-54

For purposes of this appeal, the rejections of claims 16-25, 31-44, and 51-54 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Mathies in view of McNeely and Quake and further in view of Schembri (US 6,875,620) will stand or fall together with claim 1.

2. Claims 12-15, 19, 25-35, 43-46, and 48-54

For purposes of this appeal, the rejections of claims 12-15, 19, 25-35, 43-46, and 48-54 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely and Quake and further in view of Yamamoto (US 2004/0038388) will stand or fall together with claim 1.

3. Claim 65

For purposes of this appeal, the rejection of claim 65 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely and Quake as applied to claim 64 and further in view of Klaerner (US 2002/0001845) will stand or fall together with claim 1.

4. Claim 67

For purposes of this appeal, the rejection of claim 67 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely and Quake as applied to claim 62 and further in view of Zare (US 2003/0062310) will stand or fall together with claim 1.

5. Claims 78-90, 93, 94, 96, 97, and 106-110

For purposes of this appeal, the rejections of claims 78-90, 93, 94, 96, 97, and 106-110 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely and Quake as applied to claim 1 and further in view of Werner (US 2002/0168652) will stand or fall together with claim 1.

6. Claims 91 and 98-105

For purposes of this appeal, the rejections of claims 91 and 98-105 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely, Quake and Werner as

applied to claims 87 and 89, and further in view of Christel (US 6,368,871) will stand or fall together with claim 1.

7. Claims 92 and 95

For purposes of this appeal, the rejections of claim 92 and 95 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mathies in view of McNeely, Quake, and Werner as applied to claims 90 and 93, and further in view of Regnier (US 6,156,273) will stand or fall together with claim 1.

* * * * *

For all of the foregoing reasons, Appellants submit that the Examiner's rejections of claims 1-114 as obvious under 35 U.S.C. § 103(a) in light of the cited prior art are in error and should be reversed. As set forth above, after conceding that combining the Mathies and McNeely references would not address every element of Appellants' claims, the Examiner then proposed to cure this acknowledged deficiency by combining these references with the Quake reference. But in so proposing, the Examiner ignored that the Mathies-McNeely combination teaches away from the Quake reference **and** away from the claimed invention, and the Examiner's reference combination is thus contrary to black-letter obviousness law and cannot support a *prima facie* case of obviousness.

Further, even if the Examiner's reference combination were proper, the Examiner nevertheless failed to address Appellants' objective evidence that the performance of the claimed invention is superior to the prior art by two orders of magnitude, and evidence of this degree has been held sufficient to overcome a prima facie case of obviousness. Accordingly, because the Examiner both misapplied controlling law and failed to properly consider the evidence of record, the rejections should be vacated and all claims should be allowed.

Respectfully submitted,

Date: May 15, 2009

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8. CLAIMS APPENDIX

1. (Previously presented) A microfluidic chip, comprising:

a plurality of vias;

a functionalized porous polymer monolith capable of being in fluid communication with at least one of said vias;

a microarray capable of being in fluid communication with said functionalized porous polymer monolith,

the microarray situated within at least one microchannel, the microchannel being characterized as having depth in the range of from about 1 micron to less than 10 microns, and;

an observation port through which at least one target disposed within said microarray is capable of being detected.

2. (Original) The microfluidic chip of claim 1, wherein said microarray comprises at least one probe.

3. (Original) The microfluidic chip of claim 2, wherein said probe is capable of binding said at least one target.

4. (Original) The microfluidic chip of claim 3, wherein said at least one target comprises a nucleic acid, a protein, an antigen, an antibody, or any combination thereof.

5. (Original) The microfluidic chip of claim 4, wherein said nucleic acid comprises RNA, DNA, LNA, PNA, HNA, or any combination thereof.

6. (Original) The microfluidic chip of claim 4, wherein said nucleic acid of said target is capable of hybridizing with a nucleic acid of said probe.

7. (Original) The microfluidic chip of claim 6, wherein said nucleic acid of said target comprises cDNA.

8. (Original) The microfluidic chip of claim 4, wherein said nucleic acid comprises an oligonucleotide.

9. (Original) The microfluidic chip of claim 4, wherein said nucleic acid comprises a single stranded nucleic acid, a double stranded nucleic acid, or any combination thereof.

10. (Original) The microfluidic chip of claim 5, wherein said DNA is cDNA.

11. (Original) The microfluidic chip of claim 3, wherein said microarray comprises a plurality of probes capable of binding a plurality of targets.

12. (Original) The microfluidic chip of claim 11, wherein said microarray comprises at least about 1,000 probes.

13. (Original) The microfluidic chip of claim 11, wherein said microarray comprises at least about 5,000 probes.

14. (Original) The microfluidic chip of claim 11, wherein said microarray comprises at least about 10,000 probes.

15. (Original) The microfluidic chip of claim 11, wherein said microarray comprises up to about 50,000 probes.

16. (Original) The microfluidic chip of claim 11, wherein said plurality of probes comprises at least one probe different than the other probes.

17. (Original) The microfluidic chip of claim 16, wherein said probe different than the other probes is capable of binding at least one target different than the other targets.

18. (Original) The microfluidic chip of claim 17, wherein each of said probes is individually capable of binding a target different than the other targets.

19. (Original) The microfluidic chip of claim 2, wherein said at least one probe is disposed as at least one spot on the surface of a base substrate.

20. (Original) The microfluidic chip of claim 19, wherein said at least one spot is at least about 10 microns wide.

21. (Original) The microfluidic chip of claim 19, wherein said at least one spot is at least about 20 microns wide.

22. (Original) The microfluidic chip of claim 19, wherein said at least one spot is at least about 40 microns wide.

23. (Original) The microfluidic chip of claim 19, wherein said at least one spot is at least about 60 microns wide.

24. (Original) The microfluidic chip of claim 19, wherein said at least one spot is at most about 250 microns wide.

25. (Original) The microfluidic chip of claim 11, wherein said plurality of probes are disposed as a plurality of spots on the surface of a base substrate.

26. (Original) The microfluidic chip of claim 25, wherein said plurality of spots are each separated from one another by at least about 10 microns.

27. (Original) The microfluidic chip of claim 25, wherein said plurality of spots are each separated from one another by at least about 20 microns.

28. (Original) The microfluidic chip of claim 25, wherein said plurality of spots are each separated from one another by at least about 50 microns.

29. (Original) The microfluidic chip of claim 25, wherein said plurality of spots are each separated from one another by at least about 100 microns.

30. (Original) The microfluidic chip of claim 25, wherein said plurality of spots are each separated from one another by at most about 500 microns.

31. (Original) The microfluidic chip of claim 11, wherein said plurality of probes comprise an ordered arrangement.

32. (Original) The microfluidic chip of claim 31, wherein said ordered arrangement comprises from one to three dimensions.

33. (Original) The microfluidic chip of claim 31, wherein said plurality of probes are linearly arranged.

34. (Original) The microfluidic chip of claim 31, wherein said plurality of probes are linearly arranged in two dimensions.

35. (Original) The microfluidic chip of claim 33, wherein said plurality of probes are disposed as a plurality of spots on the surface of a base substrate.

36. (Original) The microfluidic chip of claim 35, wherein said plurality of spots are disposed within at least one microchannel.

37. (Original) The microfluidic chip of claim 36, wherein said at least one microchannel varies in direction along said surface of the substrate.

38. (Original) The microfluidic chip of claim 37, wherein said microchannel is from about 10 microns to about 500 microns wide and from about 1,000 microns to about 1,000,000 microns long.

39. (Original) The microfluidic chip of claim 37, wherein said at least one microchannel is disposed as a spiral path, a serpentine path, a curved path, a straight path in fluid communication with at least one other path, or any combination thereof.

40. (Original) The microfluidic chip of claim 39, wherein said serpentine path comprises a circular serpentine path, a rectangular serpentine path, or any combination thereof.

41. (Original) The microfluidic chip of claim 40, wherein a first section of said serpentine path is disposed adjacent to a second section of said serpentine path, the first and second sections being separated by a wall of non-zero thickness.

42. (Original) The microfluidic chip of claim 41, wherein the thickness of said wall is in the range of from about 10 microns to about 1,000 microns.

43. (Original) The microfluidic chip of claim 31, wherein said plurality of probes are planarly arranged in two dimensions.

44. (Original) The microfluidic chip of claim 43, wherein said plurality of probes are disposed as a plurality of spots on the surface of a base substrate.

45. (Original) The microfluidic chip of claim 44, wherein the plurality of spots are arranged in rows and columns, said rows and columns each numbering from about 10 to about 1,000.

46. (Original) The microfluidic chip of claim 44, wherein said plurality of spots are disposed within a microwell.

47. (Original) The microfluidic chip of claim 31, wherein said plurality of probes are spatially arranged in three dimensions.

48. (Original) The microfluidic chip of claim 11, wherein said plurality of probes comprise a disordered arrangement.

49. (Original) The microfluidic chip of claim 48, wherein said plurality of probes are disposed as a plurality of spots on the surface of a base substrate.

50. (Original) The microfluidic chip of claim 49, wherein the mean distance between the plurality of spots is in the range of from about 10 to 500 microns.

51. (Original) The microfluidic chip of claim 19, wherein said probe is capable of binding said at least one target, and said spot comprises at least about one monolayer of said probe.

52. (Original) The microfluidic chip of claim 51, wherein said probes comprise nucleic acids capable of hybridizing with said at least one target.

53. (Original) The microfluidic chip of claim 19, wherein said at least one probe is covalently bonded to said substrate.

54. (Original) The microfluidic chip of claim 53, further comprising a linker molecular covalently bonded between said probe and said substrate.

55. (Original) The microfluidic chip of claim 11, wherein said plurality of probes comprise nucleic acids, proteins, antigens, antibodies, or any combination thereof.

56. (Original) The microfluidic chip of claim 55, wherein said nucleic acids comprise RNA, DNA, LNA, PNA, HNA, or any combination thereof.

57. (Original) The microfluidic chip of claim 55, wherein said nucleic acids comprise an oligomer.

58. (Original) The microfluidic chip of claim 55, wherein said nucleic acids comprise a single stranded nucleic acid, a double stranded nucleic acid, or any combination thereof.

59. (Original) The microfluidic chip of claim 56, wherein said nucleic acids comprise cDNA.

60. (Original) The microfluidic chip of claim 59, wherein said target comprises cDNA capable of hybridizing with said probe.

61. (Original) The microfluidic chip of claim 1, wherein said functionalized porous polymer monolith is capable of binding a nucleic acid.

62. (Original) The microfluidic chip of claim 1, wherein said functionalized porous polymer monolith comprises pores having a surface, said pores permitting fluid communication through said functionalized porous polymer monolith.

63. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith comprises a highly crosslinked polymer.

64. (Original) The microfluidic chip of claim 63, wherein said highly crosslinked polymer comprises units derived from at least one mono-ethylenically unsaturated monomer, at least one multi-ethylenically unsaturated monomer, or a combination thereof.

65. (Original) The microfluidic chip of claim 64, wherein said at least one mono-ethylenically unsaturated monomer comprises glycidyl methacrylate.

66. (Original) The microfluidic chip of claim 64, wherein said at least one multi-ethylenically unsaturated monomer comprises ethylene glycol dimethacrylate.

67. (Original) The microfluidic chip of claim 63, wherein said highly crosslinked polymer comprises units derived from a radical reaction catalyzed by UV activation of bis(2,6-D, methoxybenzoyl)-2,4,4-trimethylphenyl phosphine oxide.

68. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith comprises pores smaller than about 10 microns.

69. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith comprises a void fraction of less than about 50 percent based on volume of said functionalized porous polymer monolith.

70. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith is capable of operating at pressures between 100 and 3000 PSI in an aqueous fluid at 25°C that is communicated therethrough.

71. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith is covalently bonded to a substrate.

72. (Original) The microfluidic chip of claim 62, wherein said functionalized porous polymer monolith comprises at least one functional group for binding a sample compound.

73. (Original) The microfluidic chip of claim 72, wherein said functional group comprises an amine-containing ligand, an alcohol-containing ligand, a thiol-containing ligand or a hydrazine- containing ligand, or any combination thereof.

74. (Original) The microfluidic chip of claim 72, wherein said functional group comprises a nucleic acid, a protein, an antibody, an antigen, an amine-containing ligand, or any combination thereof.

75. (Original) The microfluidic chip of claim 74, wherein said nucleic acid comprises an oligonucleotide.

76. (Original) The microfluidic chip of claim 75, wherein said oligonucleotide comprises oligo-T.

77. (Original) The microfluidic chip of claim 75, wherein said at least one target comprises cDNA capable of binding at least a portion of said oligonucleotide.

78. (Original) The microfluidic chip of claim 1, wherein said microarray and said functionalized porous polymer monolith are disposed between a base substrate and a cover substrate.

79. (Original) The microfluidic chip of claim 78, wherein said microarray is disposed on a top surface of said cover substrate.

80. (Original) The microfluidic chip of claim 79, wherein said cover substrate comprises a region above said microarray to provide said observation port.

81. (Original) The microfluidic chip of claim 1, wherein both of said functionalized porous polymer monolith and said microarray are disposed between a base substrate and a cover substrate.

82. (Original) The microfluidic chip of claim 81, wherein said plurality of vias are disposed within said base substrate, said cover substrate, or any combination thereof, said vias being in fluid communication with said functionalized porous polymer monolith, said microarray, or both.

83. (Original) The microfluidic chip of claim 82, wherein said vias are capable of being in fluid communication with fluidic devices external to said microfluidic chip.

84. (Original) The microfluidic chip of claim 1, wherein at least one of said vias is not in fluid communication with said functionalized porous polymer monolith.

85. (Original) The microfluidic chip of claim 1, wherein said functionalized porous polymer monolith is not in fluid communication with said microarray.

86. (Original) The microfluidic chip of claim 81, wherein said base substrate and said cover substrate are at least partially bonded together at a bonding surface.

87. (Original) The microfluidic chip of claim 86, wherein said base substrate comprises at least one microfluidic structure disposed at said bonding surface.

88. (Original) The microfluidic chip of claim 87, wherein said microfluidic structure comprises a microchannel, a microwell, a reservoir, a microelectrode, a microjunction, a microsplitter, a microfilter, a microreactor, a microvalve, a microsensor, a microinjector, a micromixer, a micropump, a microseparator, a micromanifold, or any combination thereof.

89. (Original) The microfluidic chip of claim 87, wherein said functionalized porous polymer monolith is disposed within said microfluidic structure.

90. (Original) The microfluidic chip of claim 89, wherein said microfluidic structure comprises a microchannel, a microwell, a reservoir, or any combination thereof.

91. (Original) The microfluidic chip of claim 89, wherein said microfluidic structure further comprises microposts bonded between said base substrate and said cover substrate, said microposts being capable of reducing the deformation of said cover substrate disposed above said microfluidic structure, being capable of mixing fluid flowing through said microfluidic structure, or both.

92. (Original) The microfluidic chip of claim 90, wherein said microwell or reservoir further comprises a micromanifold, said micromanifold capable of equalizing the pressure distribution within said microfluidic structure.

93. (Original) The microfluidic chip of claim 87, wherein said microarray is disposed within said microfluidic structure.

94. (Original) The microfluidic chip of claim 93, wherein said microfluidic structure comprises a microchannel, a microwell, a reservoir, or any combination thereof.

95. (Original) The microfluidic chip of claim 93, wherein said microwell or reservoir further comprises a micromanifold, said micromanifold capable of equalizing the pressure distribution within said microfluidic structure.

96. (Original) The microfluidic chip of claim 94, further comprising a microfluidic injector in fluid communication with said microfluidic structure, said microfluidic injector being capable of providing a fluid plug into said microarray.

97. (Original) The microfluidic chip of claim 96, wherein said microfluidic structure comprises a microwell or reservoir, and said microfluidic chip further comprising a microchannel disposed between said microfluidic injector and said microwell or reservoir.

98. (Original) The microfluidic chip of claim 87, wherein said microfluidic structure is disposed in a region comprising a dimension perpendicular to said bonding surface, said dimension being up to about 1,000 microns.

99. (Original) The microfluidic chip of claim 98, wherein said dimension is in the range of from about 1 to about 500 microns.

100. (Original) The microfluidic chip of claim 98, wherein said dimension is in the range of from about 5 to about 250 microns.

101. (Original) The microfluidic chip of claim 98, wherein said dimension is in the range of from about 10 to about 100 microns.

102. (Original) The microfluidic chip of claim 87, wherein said microfluidic structure is disposed in a region comprising a dimension parallel to said bonding surface, said dimension being up to about 100,000 microns.

103. (Original) The microfluidic chip of claim 102, wherein said dimension is in the range of from about 10 to about 50,000 microns.

104. (Original) The microfluidic chip of claim 102, wherein said dimension is in the range of from about 50 to about 25,000 microns.

105. (Original) The microfluidic chip of claim 102, wherein said dimension is in the range of from about 100 to about 10,000 microns.

106. (Original) The microfluidic chip of claim 86, wherein said base substrate comprises a plurality of microfluidic structures in said bonding surface.

107. (Original) The microfluidic chip of claim 106, wherein said plurality of microfluidic structures comprises a microchannel, a microwell, a reservoir, a microelectrode, a monolith channel, or any combination thereof.

108. (Original) The microfluidic chip of claim 86, wherein said cover substrate comprises a region not bonded to said base substrate to provide said observation port.

109. (Original) The microfluidic chip of claim 108, wherein said region comprises an opening in said cover substrate.

110. (Original) The microfluidic chip of claim 109, wherein said opening is disposed above said microarray.

111. (Original) The microfluidic chip of claim 1, further comprising a derivatization reservoir capable of being in fluid communication with said functionalized porous polymer monolith.

112. (Original) The microfluidic chip of claim 111, wherein said derivatization reservoir comprises a functionalized porous polymer monolith for trapping target nucleic acids.

113. (Original) The microfluidic chip of claim 111, wherein said derivatization reservoir comprises an oligo (dT), a random oligo sequence, a gene family specific sequence, a protein ligand, or a protein receptor, or any combination thereof for stabilizing target nucleic acids or proteins.

114. (Original) The microfluidic chip of claim 1, further comprising one or more mobile monolith valves capable of controlling fluid flow in said microfluidic chip.

9. EVIDENCE APPENDIX

Attached herewith is a copy of the Declaration of Jason A.A. West, Ph.D., filed on March 25, 2008 pursuant to 37 C.F.R. § 1.132 and all exhibits thereto.

DOCKET NO.: SNL-0004 / SD-8433 **PATENT**
Application No.: 10/701,097
Office Action Dated: October 25, 2007

In re Application of:
Jason A.A. West, et al.

Confirmation No.: **4414**

Application No.: **10/701,097**

Group Art Unit: **1744**

Filing Date: **November 4, 2003**

Examiner: **Bowers, Nathan Andrew**

For: **MICROFLUIDIC INTEGRATED MICROARRAYS FOR BIOLOGICAL DETECTION**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF DR. JASON A.A. WEST UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Jason A.A. West, Ph.D., hereby declare the following:

1. I am a co-inventor of the above-captioned patent application ("the subject application"). My Curriculum Vitae is enclosed as **Exhibit A**. Among my credentials, I earned a B.Sc. in Toxicology and Chemistry from the University of Massachusetts, Amherst, USA and a Ph.D. in Pharmacology and Toxicology from the University of California, Davis, USA. I am a co-founder of Arcxis Biotechnologies of Pleasanton, California, USA, and am presently the Chief Technology Officer of that company.

2. I have performed microfluidics work relevant to the work described in this patent application as a post-doctoral fellow at the Lawrence Livermore National Laboratories, where I worked in close collaboration with General Electric Healthcare (formerly Molecular Dynamics) a microarray company. I continued this work as a Senior member of the Technical Staff at Sandia National Laboratories in the Microfluidics Research Department,

which group was a worldwide leader in the field of microfluidics. After completion of various microfluidics prototype systems, Arcxis Biotechnologies was founded to move the research into the product phase.

3. It is my understanding that claims 1-114 of the subject application are directed toward microfluidic chips.

4. I have reviewed the Office Action dated January 3, 2008. As I understand it, the Examiner has rejected claim 1 and those claims that depend from claim 1 for allegedly being obvious in light of the prior art, in particular U.S. Application No. 2004/0209354 to Mathies, U.S. Application No. 2004/0037739 to McNeely; and U.S. Pat. No. 6,833,242 to Quake.

5. This declaration is made to demonstrate that microfluidic chips recited in claim 1 of the subject application were not obvious at the time that the application was filed.

6. Based on my experience in the field of microfluidics and my own observations and tests, the invention of the instant application achieves results that are unexpectedly superior to those of alternative devices in the field.

7. Oligonucleotides processed by the claimed invention achieved in only **several minutes** a level of hybridization to target probes that oligonucleotides processed by alternative products required **several hours** to achieve. This is shown in the attached **Exhibit B**, which compares the performance of the invention that is the subject of this patent application with an existing alternative product. Also attached, as **Exhibit C**, is a manuscript that demonstrates the ability of the invention that is the subject of this patent application to perform hybridizations in only twenty minutes. In my view, this performance would not have been predicted by one of ordinary skill in the art at the time of invention.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: March 25, 2008

/Jason A.A. West, Ph.D./
Jason A.A. West, Ph.D.

Attachments:
Exhibit A
Exhibit B
Exhibit C

EXHIBIT A

Dr. Jason A.A. West
3514 Kings Canyon Ct.
Pleasanton CA 94588
(P) 925-989-7496
drjayman@gmail.com

Experience:

2003-Present **Founder**
Chief Technology Officer, Sr. Director of Research and Development
Arcxis Biotechnologies, Pleasanton, CA. 94566

Principal investigator: Phase I/II SBIR, BioPhalanx™, Hand-held instrument and consumable platform for biological agent detection.
Principal investigator: Commercial development of the Lysix™ Platform for automated nucleic acid and protein purification.
Principal investigator: Development of Altix™ Platform, a microfluidic integrated microarray system including instrumentation.
Principal investigator: Tentacle Probes™, molecular diagnostic reagents.
IP manager: Preparation and filing of Patents and Freedom to Operate opinions
Fundraising: Completed Seed round, grant funding and Series A/A1 financing.

2001-2005 **Senior Member Technical Staff**
Sandia National Laboratory, Livermore, CA. 94551-0969

Principal investigator: Design and fabrication of a microfluidic device integrated with a gene arrays for the detection of genetically engineered biological agents.
Principal investigator: Development of an unattended water sensor (UWS) for protein toxins and bacteria in municipal water supplies,
Team Leader: Directing development of Sandia's μChemlab project, focused on the detection of biological toxins and pathogenic agents.

2000-2001 **Post-Doctoral Scientist**
Lawrence Livermore National Laboratory, Livermore CA. 94550

Coordinate studies using AMS and Microarrays. Chemoprevention of Prostate Cancer; Studies based on gene expression patterns after DNA damage and alterations metabolic activation of pro-carcinogens in rats and humans.

1995-2000 **Research assistant**
University of California, Davis, School of Veterinary Medicine: APC
Specialization: Development of metabolic tolerance in response to repeated acute xenobiotic exposure

1994-1995 **Staff Scientist**
Energy and Environmental Engineering, Somerville, MA 02143
Duties: R&D- HPLC method development for quantitative analysis of soil/water/TCLP matrices for phenoxy acid herbicide and explosives content.

1992-1994 **Laboratory Technician**
Massachusetts Pesticide Analysis Laboratory, Amherst, MA 01003
Duties: Matrix extraction, sample preparation, GC, HPLC, analysis of pesticide and herbicide/pesticide residues.

Technical Skills:

Analytical: GC, HPLC, IEC, spectrophotometric, spectrofluorometric, atomic absorption spectrophotometric analysis; Method development for analysis of mammalian drug/toxicant and cellular metabolism processes, including use of radioactive compounds; Analysis of chemical residues in environmental samples, including herbicides, pesticides, and explosives; Peptide chromatography, Capillary electrophoresis, microfluidics, photo-castable polymer chemistry.

Mol. Bio.: Microarray analysis, real-time PCR, Protein/nucleic acid extraction, isolation, and blotting, protein activity measurement, transport assay development, primary cell isolation and culture, bacterial culture, drug metabolism analysis, tissue microdissection.

Microscopy: Epifluorescent and confocal microscopy, light microscopy, high-resolution histopathology, immunohistochemisty and cytochemistry, scanning electron microscopy, *In Situ* PCR, semi-quantitative fluorescent image analysis.

Computer: Apple and IBM Platforms, Web page design and construction, comprehensive use of various imaging and graphics design programs. Extensive use of Microsoft Office suite as well as MS Project, MS Visio. working knowledge of Solidworks and Alibre CAD modeling software.

Education

Doctorate: Ph.D. Pharmacology and Toxicology (2000)
Pharmacology and Toxicology Graduate Group
University of California, Davis, CA 95616

Dissertation: "Shifts in Clara Cell Susceptibility to Chemical Mediated Injury: Mechanistic Studies of Phase II Metabolic Processes Resulting in Tolerance to Bioactivated Toxicants"

Undergraduate: BS Environmental Science (1994)
Minor: Chemistry
Department of Environmental Science
University of Massachusetts, Amherst, MA 01003

Ongoing and former projects:

- Development of real-time PCR reagent platform (Tentacle Probes) for infectious disease testing, including HSV I/II, Sepsis, and bioterror agents.
- Commercialization of an integrated sample preparation technologies (Lysix™), and a microfluidic microarray platform (Altix™).
- Principal Investigator for the the Phase I and II SBIR, BioPhalanx™, an Integrated diagnostic Platform for infectious disease and biological weapons.
- Development of a microfluidic integrated microarray for the detection of genetically engineered biological agents.
- Optimization of microchannel electrophoretic protein separation and detection of biotoxins on microfluidic devices.
- Development and optimization of μChemlab methods for the detection and identification of bacterial and viral agents.

Collaborations:

- Kaiser Permanente Clinical Diagnostic Laboratory (2006- Present)
- California Department of Health, Viral Disease and Rickettsia laboratory (2005-Present)
- Arizona State University Harrington, Department of Bioengineering (2005-present)
- Sandia National laboratories (2005- Present)
- United States Army Research Institute of Infectious Disease (2003-present)
- University of California Davis, Microarray Center (2003-present)

Professional Affiliations:

- Society of Toxicology
- American Society of Pharmacology and Experimental Therapeutics

Awards/Fellowship:

- Employee recognition award – μChemLab development, Sandia National Laboratories - 2003
- Award of excellence - μChemLab development, Sandia National Laboratories - 2003
- NIEHS Superfund fellowship (1999)
- Ralph Kitchell Fellowship – Innovative teaching project (1998)
- Jastro Shields Fellowship – Academic (1996/1997)

Extracurricular activities:

- Valley Spokesman Racing Team (cycling: 2000-present)
- Team in Training- a Leukemia Society of America Fundraiser
- Rowing coach – University of California, Davis men's varsity crew (1995-2000)

Peer-Reviewed Publications:

- Brent C. Satterfield[a,b], Matt Bartosiewicz[a], **Jay A.A. West**[a] (corresponding author) (2008) *Revolutionizing detection and typing of polymorphic organisms and G-T differentiation through the use of cooperative probe technologies* (manuscript in preparation)

- Brent C. Satterfield, Matt Bartosiewicz, **Jay A.A. West*** (corresponding author), Michael R. Caplan. (2008) *Surpassing specificity limits of nucleic acid probes via cooperativity* (manuscript in preparation)

- Brent C. Satterfield[a], Matt Bartosiewicz [a], Ivy C. Yeung [b], Mark J. Stanley [b], **Jay A.A. West**[a*] (corresponding author) (2008) *Improving typing of polymorphic organisms through the use of cooperative probe technologies*(manuscript in preparation)

- Brent C. Satterfield, Michael R. Caplan*(corresponding author), **Jay A. A. West** (2008) *Tentacle Probe sandwich assay in porous polymer monolith improves specificity, sensitivity and kinetics. (*Accepted with revisions at Nucleic Acids Research)

- Brent C. Satterfield, David A. Kulesh, David A. Norwood, Leonard P. Wasieloski, Jr, Michael R. Caplan[a], **Jay A.A. West***, (2007). "*Tentacle Probes: differentiation of difficult single-nucleotide polymorphisms and deletions by presence or absence of a signal in real-time PCR.*" Clin Chem **53**(12): 2042-50.

- Brent C. Satterfield, Seth Stern, Michael R. Caplan[b], Kyle W. Hukari[a], and **Jay A.A. West*** (corresponding author) (2007). "*Microfluidic purification and preconcentration of mRNA by flow-through polymeric monolith.*" Anal Chem **79**(16): 6230-5.

- Brent C. Satterfield, **Jay A.A. West*** (corresponding author), Michael R. Caplan, *Tentacle probes: eliminating false positives without sacrificing sensitivity.* Nucleic Acids Research, 2007, Vol. 35, No. 10

- ***Jay A. A. West** and Brent C. Satterfield. Fabrication of Porous Polymer Monoliths in Microfluidic Chips for Selective Nucleic Acid Concentration and Purification. Chapter 2,* Microchip-based assay systems : methods and applications. Humana Press, Editor: Pierre N. Floriano, 2007.

- **Jay A.A. West** Kyle W. Hukari, Kamlesh Patel, Ronald F. Renzi,. Rapid Universal Solublization and Analysis of Viruses and Bacteria using a Simple Flow-Through Ultra-High Temperature Capillary Sample Preparation Device. - *2008 – manuscript in preparation to Biochemical and Biophysical research communication.*

- Ronald Renzi, James Stamps, Victoria VanderNoot, Brent Horn, **Jay A.A. West**, Boyd J. Wiendenman, Robert Crocker, Scott Ferko, and Julia Fruetel. Hand-Portable Micro Analytical Instrument for Reusable Chip-Based Electrophoresis. Part 1: System Design and Integration - 2005 – Analytical Chemistry **77**: 435-441.

- Julia A. Fruetel, Victoria VanderNoot, Brent A Horn, **Jay A.A. West,** Ronald Renzi, Scott Ferko, James F. Stamps, Isaac R. Shokair, Daniel Yee, Robert Crocker, Boyd Wiedenman. "Chip based analysis of protein biotoxins integrated into a hand portable device" – 2005 - Electrophoresis **26**(6): 1144-54.

- **West, Jay A.A**, Laura S. Van Winkle, Dexter Morin, Chad A. Fleschner, Henry Jay Forman, Alan R. Buckpitt, and Charles G. Plopper. Repeated Inhalation Exposures of the Bioactivated Cytotoxicant Naphthalene (NA) Produce Airway Specific Clara Cell Tolerance in Mice **Cover page** (2003) *Toxicological Sciences*. 75 (1) 161-168.

- Williams, KJ; **West, JAA**; Fleschner, CA; Plopper, CG. Airway Epithelial Stress Protein Expression During Repair After Acute Clara Cell Loss and in the Development of Tolerance. 2003 submitted to *Toxicology and Applied Pharmacology* (accepted).

- Buckpitt, A., Boland, B., Isbell, M., Morin, D., Shultz, M., Baldwin, R., Chan, K., Karlsson, A. Lin, C., Taff, A., **West, J.**, Fanucchi, M., Van Winkle, L., Plopper C.G. Naphthalene-induced respiratory tract toxicity: Metabolic mechanisms of toxicity. *Drug Metabolism Reviews*. -2002- v.34, no.4, p.791-820

- **West, Jay A.A.,** Kurt J. Williams, Elina Toskala, Susan J. Nishio, Chad A. Fleschner, Henry Jay Forman, Alan R. Buckpitt and Charles G. Plopper. (2002) Induction of Tolerance to Naphthalene in Clara Cells is Dependent on a Stable Phenotypic Adaptation Favoring Maintenance of the GSH Pool. *American Journal of Pathology* 160(3): 1115-27

- **West JA**, Pakehham G, Morin D, Fleschner CA, Buckpitt AR and Plopper CG (2001) Inhaled naphthalene causes dose dependent Clara cell cytotoxicity in mice but not in rats. **Cover Page** *Toxicology and Applied Pharmacology* 173:114-119

- Plopper CG, Buckpitt A, Evans M, Van Winkle L, Fanucchi M, Smiley-Jewell S, Lakritz J, **West J**, Lawson G, Paige R, Miller L and Hyde D (2001) Factors modulating the epithelial response to toxicants in tracheobronchial airways. *Toxicology* 160:173-180.

- **West, J.A.A.**, Chichester, C.H., Buckpitt, A.R., Tyler, N.K., Brennan, P., Helton, C., and Plopper, C.G. "Heterogeneity of Clara cell glutathione: A possible basis for differences in responses to pulmonary cytotoxicants." – 2000 – *American Journal of Respiratory Cell and Molecular Biology* 23(1) pp. 27-36.

- **West, J.,** Buckpitt, A., Plopper, C., "Elevated intracellular glutathione (GSH) resynthesis confers protection to Clara cells from naphthalene (NA) injury in tolerant mice" – 2000 – *Journal of Pharmacology and Experimental Therapeutics* 294(2) pp. 516-523.

- Plopper, CG, Van Winkle LS, Fannuchi MV, Evans MJ, Weir AJ, Nishio SJ, Postlewaite ES, **West J**, Buckpitt AR, Hyde DM. "Basic Principles for Use of Fluorochromes and Filters." *Faseb Journal* 14 (4): A17 Mar 15 2000.

Patents and technical advances:

- *Polymer Microfluidic Biochip Fabrication.* Jesse Thompson, Jay A.A. West. – In Preparation - PCT Patent App. (2007)

- *PCR-free sample preparation and detection systems for high speed biologic analysis and identification.* Kyle W. Hukari, Brent C. Satterfield, and Jay A.A. West, PCT patent App. (2007)

- *Disposable sample preparation cards, methods, and systems thereof.* Brent C. Satterfield, Seth Stern, Michael DeRenzi, Kyle W. Hukari, and Jay A.A. West, US patent App. 60829079 (2006)

- *Cooperative probes and methods of using them.* Brent C. Satterfield, Jay A.A. West. PCT patent App. 60850958 (2006)

- *Selection of Aptamers Based on Geometry.* Brent C. Satterfield, Jay A.A. West. US provisional patent App. 60870493 (2006)

- *Apparatus and method for carrying out chemical or biological processes.* Kyle W. Hukari, and Jay A.A. West, US patent App. (2006) Patent Pending

- *A Sample Preparation Device for Integrated Lyses, Labeling and Analysis of Robust Bacteria, Bacterial Spores, and Viruses.* Jay A.A. West, Kyle W. Hukari, Kamlesh Patel, Ronald F. Renzi PCT2005034045 US60/612,969 (2005)

- *Miniaturized Microfluidic Genearray platform for fluidic handling and real-time detection of array probes.* Jay A.A. West, Kyle W. Hukari, Gary A. Hux (2005) *Patent pending*

- *High Density Specific Location Microarray Spotting Software.* Kyle W. Hukari, Jay A.A. West. (2004) *Copyright pending*

- *Viral identification by generation and detection of protein signatures.* Jay A.A. West, Todd W. Lane, James F. Stamps, Isaac R. Shokair, Julia A. Fruetel. US patent App. 20050014134. *Patent Pending.*(2004)

- *A Modular Device for Microscale Biotoxin Detection.* Julia A. Fruetel, Victoria VanderNoot, Brent A Horn, Jay A.A. West, Ronald Renzi, Scott Ferko, James F. Stamps,

Isaac R. Shokair, Daniel Yee, Robert Crocker, Boyd Wiedenman. (2003) US patent App. 20040126279 *Patent Pending*

- *Development of a microfluidic integrated microarray for detection of chimeric biological weapons.* Jay A.A. West, Boyd Wiedenman. (2003) US patent App. 20050095602. *Patent Pending.*

Conference Abstracts, Presentations, and non-refereed articles:

- BioPhalanx I: Development of Diagnostic Reagents to Provide Reliable Detection of Biological Agents Jay A.A. West, Brent C. Satterfield, Matthew Bartosiewicz, Kyle W. Hukari, Jesse Thompson, Fabian Van de Graaf -2008- Annual Chemical and Biological R&D Technologies Conference January 28 – February 1, 2008 – San Antonio, Texas

- Lysix™ MPCs (micro-purification cards) and the Lysix™ Nucleic Acid Workstation 808 Matthew Bartosiewicz, Senior Scientist, Arcxis Biotechnologies Jay West, Ph.D., CTO, Arcxis Biotechnologies, IBC Conference, September 18 - 19, 2007, Philadelphia, PA

- *BIOPHALANX: a hand portable discrete monolithic microarray Biothreat Detector* AND *Tentacle Probes: discrimination of difficult single nucleotide polymorphisms* Jay West, Arcxis Biotechnologies, Homeland Security Research Innovation & Transition Conference Monterey, CA Aug 21—24, 2007

- Arcxis Biotechnologies: Platforms for Rapid PCR-Free detection of Biological Threats. Kyle W. Hukari, Dustin Li, Mike Derenzi, Seth Stern, Quang Thai, Brent A. Satterfield and Jay A.A. West. Gordon Conference, Chemical & biological terrorism defense, Integrating Biodefense, Homeland Security And Basic Science January 14-19, 2007, Ventura, CA.

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EXHIBIT B

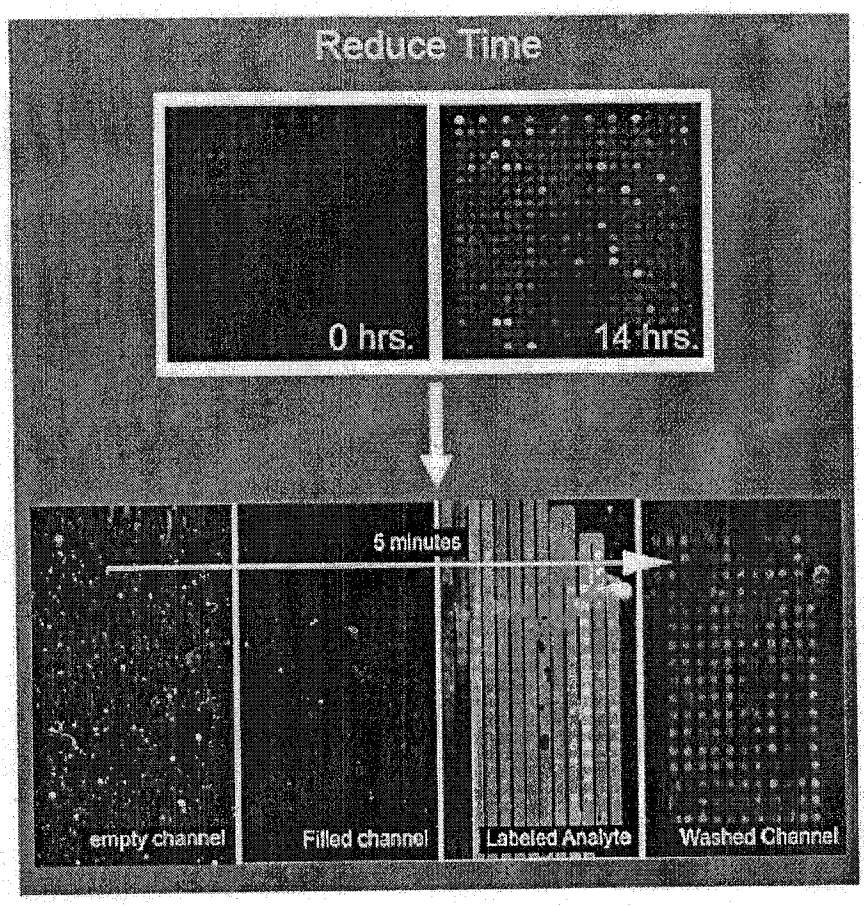
Reduce Time
0 hrs.
14 hrs.
5 minutes
empty channel
Filled channel
Labeled Analyte
Washed Channel

EXHIBIT C

Microfluidic Gene Arrays for Rapid Genomic Profiling

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Abstract

Genomic analysis tools have recently become an indispensable tool for the evaluation of gene expression in a variety of experiment protocols. Two of the main drawbacks to this technology are the labor and time intensive process for sample preparation and the relatively long times required for target/probe hybridization. In order to overcome these two technological barriers we have developed a microfluidic chip to perform on chip sample purification and labeling, integrated with a high density genearray. Sample purification was performed using a porous polymer monolithic material functionalized with an oligo dT nucleotide sequence for the isolation of high purity mRNA. These purified mRNA's can then rapidly labeled using a covalent fluorescent molecule which forms a selective covalent bond at the N7 position of guanine residues. These labeled mRNA's can then released from the polymer monolith to allow for direct hybridization with oligonucletide probes deposited in microfluidic channel. To allow for rapid target/probe hybridization high density microarray were printed in microchannels. The channels can accommodate array densities as high as 4000 probes. When oligonucleotide deposition is complete, these channels are sealed using a polymer film which forms a pressure tight seal to allow sample reagent flow to the arrayed probes. This process will allow for real time target to probe hybridization monitoring using a top mounted CCD fiber bundle combination. Using this process we have been able to perform a multi-step sample preparation to labeled target/probe hybridization in less than 30 minutes. These results demonstrate the capability to perform rapid genomic screening on a high density microfluidic microarray of oligonucleotides.

Introduction

Sensitive, accurate detection and portable identification of biological agents is critical to our ability to avert wide spread casualties from a bio-terrorist threat. The use of Gene or "micro" arrays has become a widespread bio-analytical technique for genomic profiling. Recent advances in microarray technology have led to diagnostic applications that are currently being developed for the detection of biological pathogens. Two major drawbacks to the current experimental format in which these assay are performed are the degradation sensitive long sample processing time required for probe generation and the extended time requirement for the aforementioned probes to be hybridized to the target DNA on the slide surface. The goal of these studies was to develop an array based microfluidic chip and platform that can be adapted to a hand portable device. We have also focused our efforts to develop a platform that is easily customized for rapid assay development in a wide application space. This will be accomplished by integrating two newly emerging and maturing technologies: microarrays and microfluidics. Microarray devised devices were first described in 1989 by Ekins et. al. [1] where antibodies were affixed to a solid substrate. Subsequent publications Fodor [2] and Schena [3] set the stage for the explosion of *DNA, gene* or *Micro* array technology. Since these landmark publications, various chip design platforms including oligonucleotide, EST, and cDNA have been developed along with an ever expanding number sources of *content* or synthesized genes for detection of a particular agent or expressed gene. These array technologies vary widely in their format as well as their application. High density oligonucleotide arrays, based on Fodor's publication [2] later became the base technology for Affymetrix (patent # *5,445,934*). These oligonucleotide formats have extremely high density, with the capacity to perform measurements on over 350,000 DNA elements in a single experiment. While this technology has great measurement capacity, due the photolithographic manufacturing process using multiple masks, it is less well suited for integration with microfluidic systems. A better suited microarray platform for microfluidic integration in a custom or robotically spotted cDNA microarray first described by Schena [3].

This array format has demonstrated good correlation to standard quantitative genetic assays such as northern blot analysis [4]. Additionally, using a robotic spotter for array construction offers the capability to deposit high density DNA probes in complex architectures, such as microfluidic systems that also offer the flexibility to routinely alter content of the device to optimize assay conditions rapidly. Low density microarrays have also been recently been developed [5-7]. These devices make use of a plastic microfluidic channel to effect rapid target to probe hybridization times. The current studies were designed to extend this capability to develop on-chip sample purification and rapid sample labeling. We also aimed to produce a device that can perform these rapid hybridization reactions in a high density microarray format.

In order to realistically manipulate this technology for practical purposes outside the laboratory, it is crucial to first determine the most efficient method of hybridizing a target sequence to a known probe sequence. In these experiments, different techniques of labeling mRNA and cDNA, including those with Cye dyes and Universal Labeling System (ULS) dyes, were performed. In order to further increase the speed in which these type of analyses can be performed, we have designed and fabricated a microfluidic microarray that will both decrease sample probe preparation time as well as target to probe hybridization time. The development of such devices will allow for the miniaturization of microarray instrumentation and make hand-portable microarray techonlogies a reality. We first focused on the fabrication of the high density microfluidic microarray that has the ability to detect thousands of individual elements in real-time. We have optimized the design of this chip to affect reduced hybridization times of target oligonucleotides, eliminate diffusion effects to optimize chip-based nucleic acid hybridizations, and to maximize signal to noise ratios in order to reduce the optical limit of detection. We have further developed porous polymer monolith material for the trapping of mRNA. Using this polymer we demonstrate the rapid trapping and release of purified mRNA. To reduce the time required for sample processing, we evaluated several commercial nucleic acid labeling protocols. These experiments demonstrate that it is possible to generate hybridization ready samples in less than twenty minutes. Finally we have developed the first generation fluidic and detection platform. This platform will allow for easy use of the fabricated microfluidic chips, and is capable of both performing fluidic control elements as well as optical detection. We are currently integrating thermo-controlling capability into the device. In this manuscript we demonstrate the individual processes, such as sample trapping and rapid hybridization, in order to translate these technologies to a completely integrated hand-portable device.

Materials and Methods

Reagents and supplies: The following chemicals were used at various steps of experimentation and were used without further purification. 3-Glycidaloxypropyldimethoxymethylsilane, 99% pure Hexanes, Ethanolamine, sodium dodecylsulfate (SDS), NaCl, and Ethanol were purchased from Aldrich Chemical Co. DEPC water, 20X SSC, 20X SPPE buffers were purchased from Invitrogen. Rat oligo test set, spotting buffers, and epoxy coated microscope slides were purchased from MWG. PCR clean-up kits were purchased from Qiagen. The microfluidic manifold was constructed on site using Delrin and aluminum stock. O-rings were purchased from Apple Rubber. Microfluidic fittings made with PEEK were supplied by Sandia National Labs. Ulysis DNA labeling kits were purchased from Molecular Probes (Eugene, OR.). CyScribe kits for first strand synthesis and cytosine labeled Cye dyes were purchased from Amhersam biosciences. All other chemicals purchased were reagent grade or better.

Microfluidic chip fabrication: Devices were optimized by experimental modeling and fabricated using standard photolithography techniques. Microfluidic chips were fabricated in fused silica which has the advantage of detecting fluorescent spots with a high signal/noise ratio [8]. Fabrication with fused silica allows for the use of multiple etch depths to normalize pressure gradients and reduce the diffusion distance for target analytes to the probe surface. This reduces hybridization time dramatically. Integration of microarrays with a microfluidic device was accomplished by designing and fabricating areas for performing sample clean-up, amplification and/or hybridization on a single chip. The construction of these arrays was accomplished using a multiple depth photolithographic etch process on two separate four inch quartz wafers which were subsequently bonded at 1100°C.

Spotting of oligonucleotide probes to the microfluidic microarray chips is achieved by use of a robotic spotter that deposits the probes on to a custom addressable array. The robotic spotter has micron resolution to position the spotting pens within the microchannels. Further details of robotic spotting are provided in U.S. Patent Application Serial No. 10/701,097, "Microfluidic Integrated Microarrays for Biological Detection", filed November 4, 2003. After spotting, the microfluidic chip is cleaned and prepped following standard procedures (0.1% sarcosine, next 3x SSC, rinse in deionized water, and immersion in ice cold ethanol). Sample oligonucleotide probes, for example commercially-

available rat DNA probes from MWG Biotech (High Point, North Carolina) are attached to the microarray surface according to these processes.

Cye Labeled ULS and Labeled ULS mRNA: To evaluate different methods of analyzing gene expression (mRNA) first strand synthesis (mRNA to cDNA) was executed with and without incorporating a Cy labeled d(CTP). Unlabeled cDNA was made using 1.0 ul 1 mM d(CTP) instead of the Cy dCTP. The reaction mix (Amersham Biosciences) was then added to the mRNA mix at room temperature. After 10 minutes 1.5 ul of reverse transcriptase (RTase) was added to the mixture, and then placed at 42°C for 1 hour. The reaction was stopped by snap cooling on ice for 30 seconds. 1.0 ul of RNase H and 0.5 ml RNase cocktail (A+T1) (Stratagene) was added to degrade the mRNA. The reaction was followed by purification using a PCR clean up kit (Qiagen) with spin columns. The unlabeled cDNA and mRNA were labeled using various Universal Labeling System (ULS) dyes which utilize a platinum complex that attaches to the the N7 position of Guanine in nucleic acids. 1.0 ug of mRNA was added to 19 ul of the labeling buffer and denatured at 95°C. The cDNA was incubated for 15 minutes at 80°C, and the mRNA incubated for 10 minutes at 90°C. Concentrations and base/dye ratios were determined using a microplate reader.

Monolith Development:
The synthesis of suitable probes for microarray analysis depends on the isolation of high quality RNA or DNA samples, efficient labeling of these purified samples, and removal of unbound dye prior to probe hybridization. Using UV initiated porous polymer monolith we are able to selectively pattern nucleic acid purification columns in microfluidic chips. Once polymerized, these monolith columns are post functionalized with a variety of amine terminated oligonucleotides which traps the targeted nucleic acids. Such monoliths are especially well adapted to microfluidic devices, as they can be polymerized in any particular pattern, have a selectable porosity, and high surface area for efficient nucleic acid binding. Trapping of target oligonucleotides was accomplished using a UV cured porous polymer monolith. Chips are pretreated overnight with a 10 parts water, 6 parts acetic acid, and 4 parts z-6030 solution. Chips are then flushed with filtered buffer and dried. To Prepare the monomer solution; add to a 7-mL vial, (added in this order:) 1940 uL of methanol, 660 of ul ethyl acetate, 840 uL of GMA, 560 uL EGDMA, and 8 mg of Irgacure 1800 were combined and vortexed to mix thoroughly. Using a hood vacuum and nitrogen supply, the monomer solution was purged 3 times to remove the oxygen, then sonicated under a vacuum to remove gases. The fluidic chip was filled with monomer solution (flush with at least 10 volumes), taking care to avoid bubble formation. The free monomers in the chip were then polymerized in a UV crosslinking oven for 30 minutes.

Fluidic hardware and detection platform development: Chip manifolds and supporting hardware were designed and fabricated to interface the microfluidic chips using an O-ring face seal. Detection of DNA microarray spots on the microfluidic chip was accomplished using a combination of light delivery using fiber waveguides with a CCD array detection platform for detection of fluorescent spots on the array surface. Using AA battery power to drive an 80mW LED, we are able to deliver light to the chip using a custom fabricated fiber bundle waveguide. This waveguide illuminates the channel where the DNA microarray is located. Oligonucleotide probes were then deposited in the open microchannels using a conventional arraying robot which was controlled by a custom script to allow the deposition of probes in specific locations. These open channels containing the covalently linked oligonuceotides were then sealed with a polymer film in order to flow reagents to each spot on the device. The microarray is sealed using pressure or adhesion with an optically transparent chemically resistant plastic film. The microarray can be adhesively sealed by treating a chemically resistant clear plastic sheet (e.g., PDMS) with plasma oxidation to activate surface functional groups. Amine-terminated silane was then applied to the plasma oxygen activated surface to enable an epoxy-amine sealing reaction between the amine coated surface of the plastic film and the epoxy coated fused silica chip. The plastic surface is then bound to the tops of the ridges between the channels and top side of the chip. Chips are housed in a manifold outfitted with o-ring seals designed to allow for one step chip alignment and to facilitate fluid connections to the outside environment. A Basler A102fc color CCD camera with an 2:1 pulled fiber optic was purchased from Videoscope International. Capillary tubing was purchased from Polymicro, inc.

Results

The microfluidic chips were designed to accommodate on-chip sample purification, labeling and array analysis. To accomplish this, specific regions for sample concentration and arraying are designed into a single device. Using this process we were able to create areas on a compact device (2.5x3.1cm), which can accommodate UV, patterned monoliths for sample processing (figure 1 B&C) and an array-spotting surface for post-fabrication DNA arraying. We designed (figure 1A) and fabricated (figure 1B) the first generation of microfludic microarrays. In this process, two wafers are etched then, bonded to produce the microfluidic chips. Fabricated Chips (Figure 1B) contained an open channel that is etched 15 um into the top surface of the microfluidic device (Figure 1B&C). This design allows spotting of DNA probes on the fabricated chip in an open channel. This architecture serves several purposes. Critical to the design of these chips is the ability to robotically spot DNA detection (probes) on the channel array surface after fabrication of the microfluidic chip. The shallow serpentine micro channel (15um height x 300 um wide) greatly reduces the hybridization time by minimizing the diffusion time of the sample and fluidically directing the target past each surface probe. The second set of channels are closed and are located between the two wafers. These channels house a monolith material that is used for sample purification.

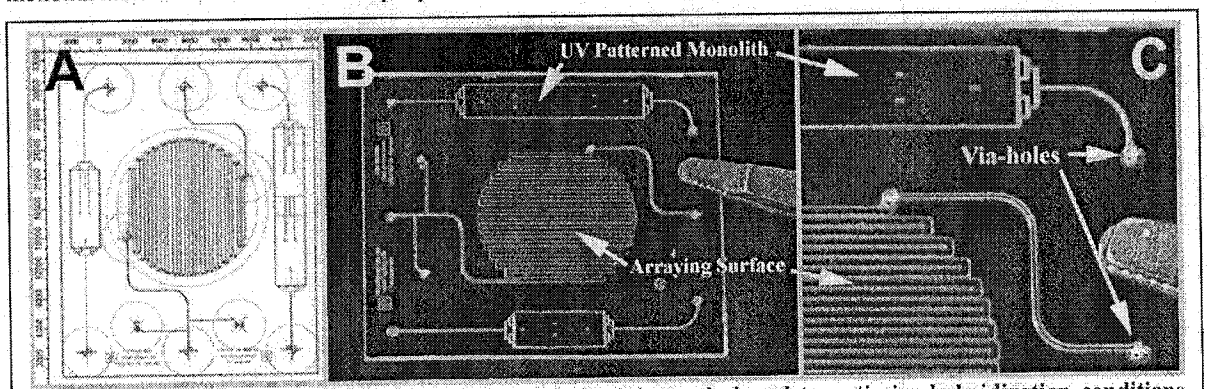

Figure 1: Fabricated microfluidic microarray. Masks (A) are designed to optimize hybridization conditions such the entire injected sample passes through the serpentine array surface (B&C) which is spotted post-fabrication. Using a double wafer etch process areas for containing monoliths are isolated from the array surface.

Monolith: The Oligonucleotide trapping monolith is composed mainly of gyclidyl methcrylate (GMA) which has a porous structure with roughly 1-3mm pores (Figure 2A). The Gycidyl (epoxide) chemistry allows for post-functionalization of the polymerized monolith, as seen in Figure 2B&C. Polymerized monolith (Figure 2B), is not fluorescent at 488nm (blue light). In contrast when this monolith is post-functionalized (C) with any amine containing molecule (Oregon Green), the monolith appears as a fluorescent signal housed in the capillary (Figure 2B).

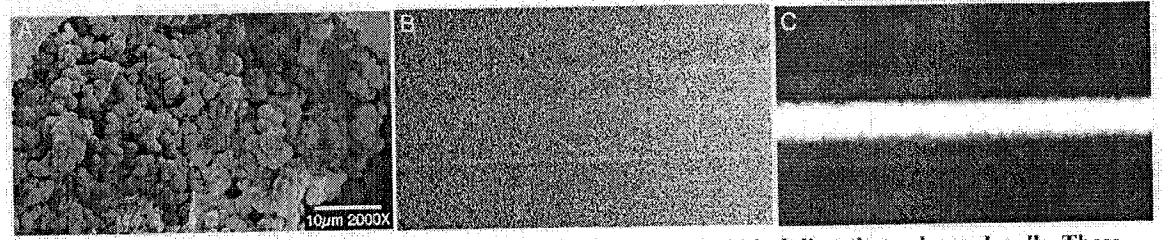

Figure 2: Porous polymer monolith are photointiated on-chip (A) and bind directly to channel walls. These formed monoliths have no native fluorescence (B), but can be post-functionalized to incorporate any primary amine containing molecule including fluorescent dyes (C), oligonucleotides, proteins, etc.

In order to trap oligonucleotitides the glycidyl methacryalte polymer was functionalized using an amine terminated oligo dT which contained a C6-linker molecule. Once the monolith was polymerized in the channel the oligo dT solution was introduced in a Tris buffer pH 8.3. The monolith was then heated to 60°C for thirty minutes in a humid chamber. Unbound oligo dT was then flushed out using additional buffer solution. mRNA's (in a TE/SSC buffer) were then isolated by using a syringe and syringe pump to deliver flow through the porous polymer monolith. The hybridization in the monolith is rapid, occurring in less than two minutes (figure 3A). After a secondary flushing step using the binding buffer, which does not result in decreased fluorescence, the mRNA was eluted using the TE buffer as seen in Figure 3B.

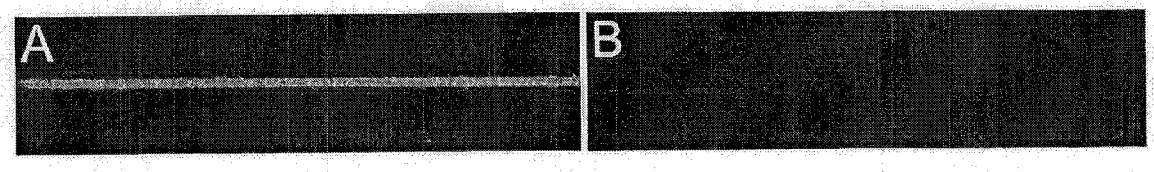

Figure 3: Oligonucleotide trapping monolith: These monoliths were functionalized with an Oligo dT. After functionalization the labeled mRNA was dissolved in the binding buffer was introduced to the column, which became bound (A). After a flushing step (not shown) the trapped oligonucleotide was eluted from the column using a salt free TE buffer. This entire process was complete in two minutes.

mRNA labeling: To determine the optimal strategy for fluorescently labeling nucleic acids we tested three commercial protocols for use with our system. Depending on the labeling strategy the the intensity of the probe hybridization on the microarrays appears to have variability, while the patterns of gene expression appear identical between labeling platforms (data not shown). In general the direct incorporation of Cye labeled oligonucleotides produced more consistent labeled cDNA's. However, as previously reported their appeared to be significant bias in the incorporation of the Cy3 vs. Cy5 dye set into the synthesized cDNA's. We also found that this labeling technique is not well suited for the labeling of oligonucleotides for the rapid detection of genetic signatures. In contrast, we found that the direct labeling of mRNA was an efficient technique for obtaining labeled full length nucleic acids. We found that the expression patterns between synthesized cDNAs (figure 4B) and direct labeled mRNA (Figure 4C) were identical. In addition, compared to the first strand (cDNA) method, the direct labeling of mRNA can be accomplished (including clean up) in approximately 10 minutes.

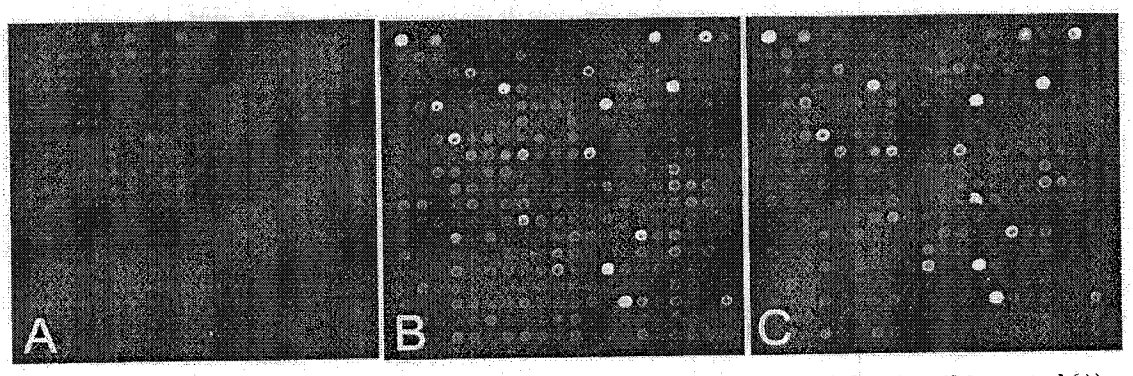

Figure 4: Comparison of first strand cDNA synthesis to direct mRNA labeling. Compared to control (A), 0.581ug alexa fluor 532 labeled synthesized cDNAs generated a robust and easily detectable signal (B). This was also the case for the direct labeled mRNAs as 0.372ug alexa fluor labeled mRNA generated a highly detectable gene expression profile (C).

Detection and Hybridization: In order to detect the hybridization of target to probe on the array surface a detection platform that utilizes a second collection fiber bundle array is place on the array surface (Figure 5A). When the microfluidic chip is illuminated the pattern of the array surface is easily observable through the fiber bundle (figure 5B).

When this collection fiber bundle is mated to a color CCD camera the CCD camera is placed adjacent to the fiber bundle which is placed on the plastic film for imaging the microarray (Figure 5A). Using this system it is possible to detect fluorescent spots on the array surface or presence of fluorescent dyes in solution in the channel. To observe the hybridization of mRNA in the microfluidic microarray chip, the chip was first spotted with oligonucleotides. The chip was then sealed using a compression plug which sealed a polymer film on the fused silica microarray chip. The labeled mRNA was pre-heated to 90°C, then introduced to the array which was held at a constant temperature of 42°C. The mRNA solution was then allowed to stand in the array channel for 5 min, after which addition aliquot of solution was passed into the array. After the second incubation period was complete the channel was imaged. We found the images collected prior to the hybridization (figure 5C) did not display any detectable mRNA target probe hybridizations. In contrast in when fluorescently labeled mRNA were passed through the channel were able to detect the hybridization of the target mRNA to the surface probe in less than twenty minutes (Figure 5D).

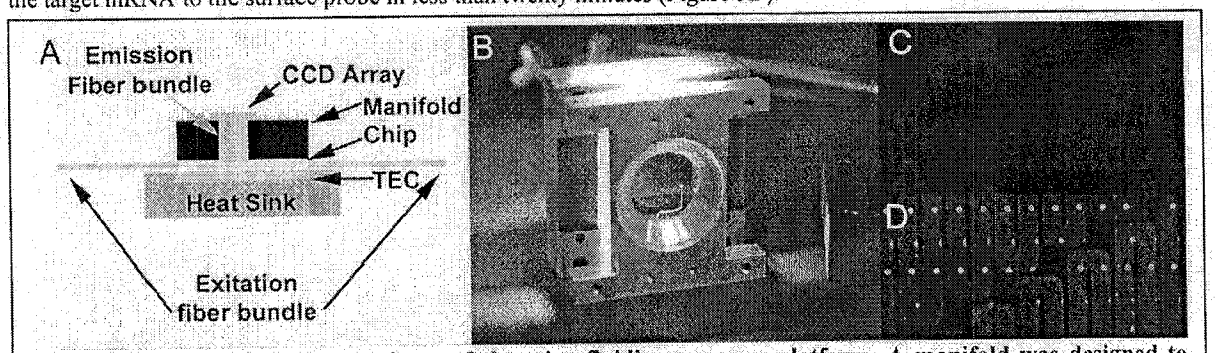

Figure 5: Design, fabrication and use of the microfluidic genearray platform. A manifold was designed to incorporate the use of excitation source, emission light collection using a CCD camera and thermocycling apparatus (A). The manifold and associated parts were fabricated to house the microfluidic chip and associated optics (B). Using this system to perform on-chip hybridizations we found mRNA labeled with Alexa fluor 647 annealed to the non-fluorescent spotted microfluidic genearray (C) in less 20 minutes (D).

Discussion:

Our results demonstrate the feasibility of fabricating high density microfluidic genearray chips with the capability of performing rapid genomic profiling. The chips are simple to use and easily customizable allowing for the rapid alteration of probe content. In addition the entire assembly is miniaturized and can be packaged in a hand-portable platform for field analysis of biological agents. Previous work by Lenigk, et.al demonstrated the feasibility of the production of microfluidic genearrays [7]. In these studies the number of probes was limited to capability of the device used to deposit oligonucleotide probes in the microfluidic channels. In the present study we have developed a custom spotting scripting software program that allows for the deposition of up to 4000 individual elements in selectable positions along the microfluidic channel. As demonstrated in the previous studies [6, 7], the use microfluidics dramatically decreases the time required for the hybridization to the oligonucleotide probes in the microchannel. In our studies we have seen hybridization times of less than twenty minutes. In addition we have developed an integrated platform for the easy use of the microfluidic chips. This system includes the development of sample trapping monolith polymers which have the ability to trap and elute fluorescently labeled mRNA in less than two minutes. These polymers were similar to those reported for use as a separation matrix for polycyclic aromatic hydrocarbons [9-11]. During the fabrication of the polymers in this study we took great care in preserving the glycidyl functionality of the polymerized monomer. After this initial polymerization a second reaction to functionalize the polymer was conducted. We found that we were able to extensively functional the surface of this polymer with any primary amine containing molecule. In this study this was limited to amine terminated fluorescent dyes, and Oligo dT. However, the flexibility of this polymer will make the functionalization of the surface chemistry capable of accepting any selective trapping molecule. Such chemistry will allow for the use of these columns as pre-selection concentrators for enrichment of a subset of genes of interest in following studies.

We further evaluated the ability to rapidly label nucleic acids using a direct labeling method. We found that the direct labeling method held several advantages over the current standard first strand synthesis of cDNAs. First, because

the reaction is simple, we found far fewer difficulties with contamination of labile mRNA samples. In addition, the method was far more rapid, only requiring a maximum of twenty minutes compared to the usual four-six hour procedure required for first strand cDNA synthesis. Finally, we found that the using this enzyme free system was far easier to integrate with our assay platform as the labeling of trapped mRNA samples can be accomplished by only changing a single solution in the polymer monolith.

Detection of the amplified microarray spots can be carried out using optical fiber bundle arrays, that both transmit and collect light. These fiber bundles deliver high intensity light from low power consuming and robust LED's. Detection is carried out with a high resolution CCD array capable of collecting signal from all elements on the microarray in parallel. The resolution of this CCD array makes it possible to detect all spots in parallel without the need for focusing optics. The CCD array/fiber bundle is be placed directly onto the arraying surface of the chip (figure 1B). This capability will make it possible to detect the presence or absence of thousands of threat genes in *real-time*.

In summary, we demonstrate a first generation integrated microfluidic high density microarry platform (up to 4000 probes), that has the ability to house rapid sample preparation, hybridization, and detection of genomic samples. This allows sample preparation of less than 40 minutes including; direct labeling of mRNA's, trapping with porous monolith and elution for hybridization. Most importantly this device demonstrates the hybridization of mRNA's in a microfluidic channel in less than 20 minutes. In the future we plan to perform real time detection of probes using a the fully integrated handheld portable device.

Acknowledgements

The authors would like acknowledge the efforts of James Stamps for his support on the electronics fabrication, Brent Satterfield for his work with the trapping monoliths, Rachel DeVay for her work with mRNA hybridizations, as well as Boyd Wiedenman and John Brazzle for their assistance with chip fabrication. We acknowledge the assistance and materials support from Dr. Micheal Shultz and Dr. Alan Buckpitt at the University of California, Davis; Toxicogenomics Center. This work was funded by a Sandia National Laboratories Laboratory Directed Research and Development grant, Project 52706 "Development of a Microfluidic Microarray for the Detection of Chimeric Biological Weapons".

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10. RELATED PROCEEDINGS APPENDIX

No related appeals or interferences are currently pending.